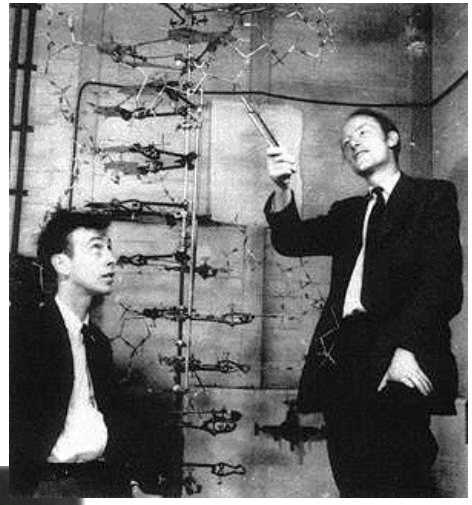
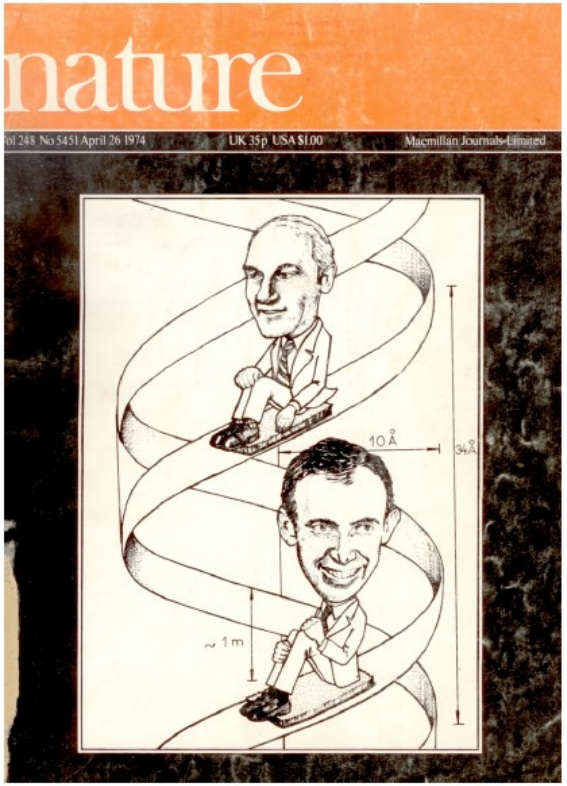
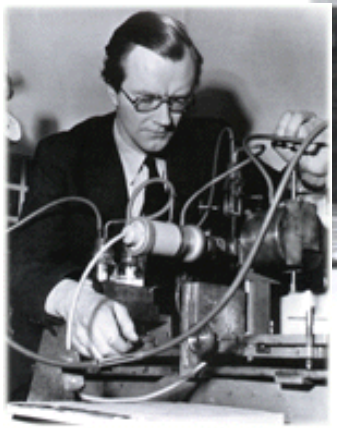
Nukleové kyseliny







1953: James Watson, Francis Crick, Rosalind Franklin, Maurice Wilkins: dvoušroubovice DNA

1962: Nobelova cena (JW, FC, MW)

vysvětlení základních principů uchování, předávání a exprese dědičné informace

Chargaff's Rules

Tetranucleotide hypothesis originated in 1906: DNA is a "statistical tetranucleotide".
During the 1950´s E. Chargaff showed a number of DNAs, which differ in their base content.
Chargaff´s rules: 1. 6-amino residues = 6-keto-residues; in another expression *A+C = G+T*;
2. py = pu; *C+T = G+A* 3. *A/T = G/C = 1* (consequence of combining equations 1 and 2)

Watson and Crick (1953) proposed their famous double-helical structure of B-form of DNA on the ground of Chargaff´s rules

- X-ray diffraction of DNA fibers obtained by Maurice Wilkins and Rosalind Franklin
- Construction of molecular models

This structure consists of two antiparallel helical strands. One turn contains 10 residues in every strand, the distance between bases is 3.4 *A*, the bases are almost perpendicular to the axis, the phosphate group is 9 *A* from the axis. Bases are specifically paired through hydrogen bonds – *AT* and *GC*. The strands are complementary – hydrogen bonds between two strands, the bases are inside the structure. Difference from α-helix in polypeptides. Further forms *A* and *C* (besides B): dependence on humidity. The differences are principally in the tilt of bases and in the number of residues per turn, strands are commonly antiparallel, bases are stacked and base pairs located in one plane. It seems that the B-form is the prevalent one in solution as well as in cells and viral particles.

Crick, Watson and Wilkins: Nobel Prize 1962

"The structure is produced like a rabbit out of a hat, with no indication as to how we arrived at it"
F. Crick, NATURE 248(1974) 766- on the occasion of the 21st anniversary of the discovery (commenting their first paper in NATURE). What experimental evidence was available to W+C in 1953?

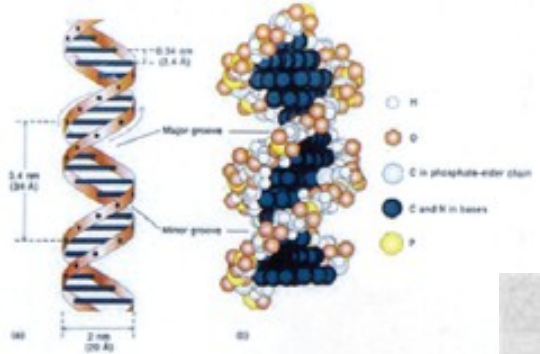
X-RAY FIBER ANALYSIS OF DNA

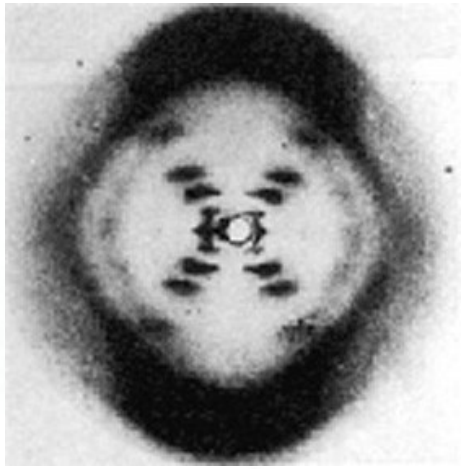
represented the main evidence for the Watson-Crick double helix model

This method enabled analysis of high-molecular DNA, but provided only few basic parameters of the helix, such as

distance between base pairs

number of base residues per turn

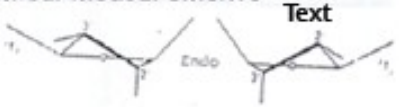




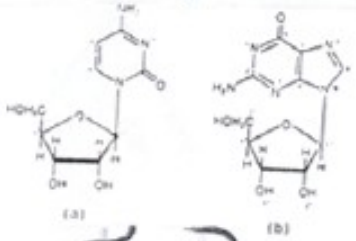
Further data were derived from model building considering the laws of structural chemistry

Base pairing from physical-chemical measurements

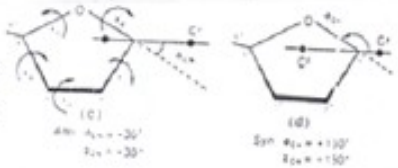
Text



Sugar configuration (PUCKER)

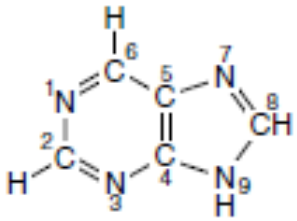


Angles of the glycosidic bonds were fixed within certain limits

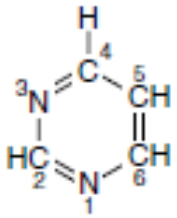


Handedness of the helix

The direction of rotation was guessed and then subjected to testing

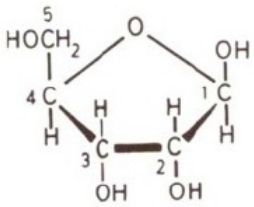


Purine

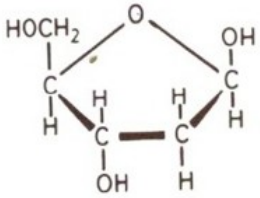


Pyrimidine

Figure 33–1. Purine and pyrimidine. The atoms are numbered according to the international system.



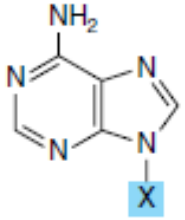
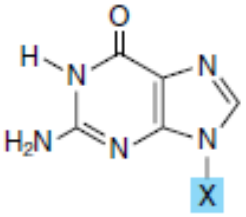
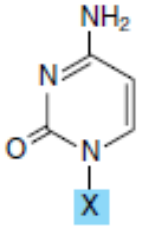
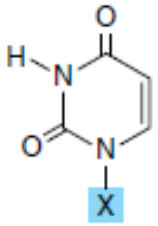
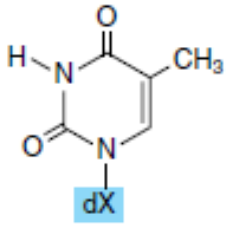
β-D-ribofuranose



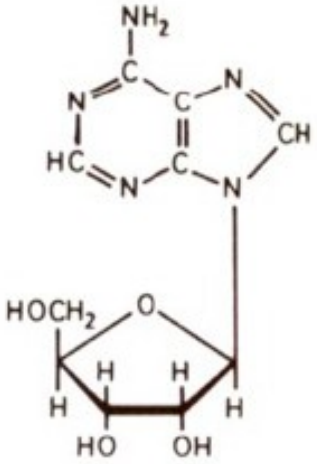
β-D-2-deoxyribofuranose

Číslovaní **uhlíků** ve zbytcích **cukru**:
1', 2' ....5' na rozdíl od bází 1, 2, ... 5, 6 ..

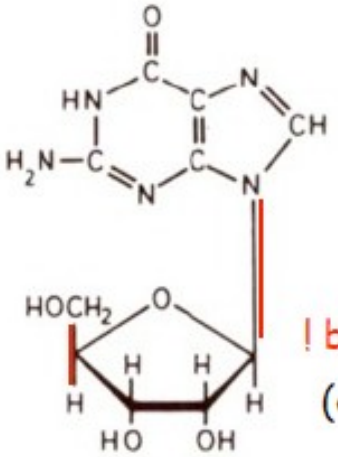
Sugar puckering

Base Formula	Base X = H	Nucleoside X = Ribose or Deoxyribose	Nucleotide, Where X = Ribose Phosphate
	Adenine A	Adenosine A	Adenosine monophosphate AMP
	Guanine G	Guanosine G	Guanosine monophosphate GMP
	Cytosine C	Cytidine C	Cytidine monophosphate CMP
	Uracil U	Uridine U	Uridine monophosphate UMP
	Thymine T	Thymidine T	Thymidine monophosphate TMP

Nucleosides/nukleosidy



Adenosine
(9-β-D-ribofuranosyl adenine)



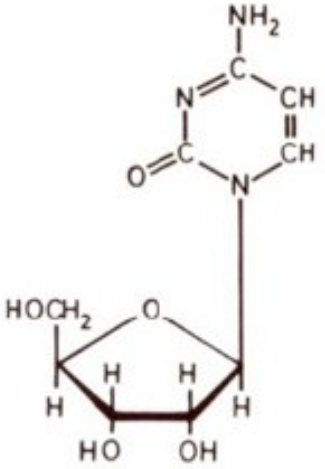
Guanosine
(9-β-D-ribofuranosyl guanine)

! beta-glykosidická vazba

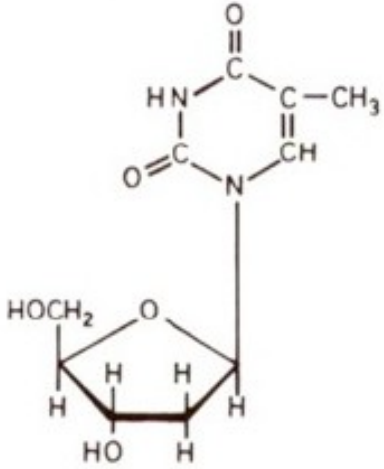
(obě vazby na stejné straně kruhu cukru)

Guanine riboside

Guanosine



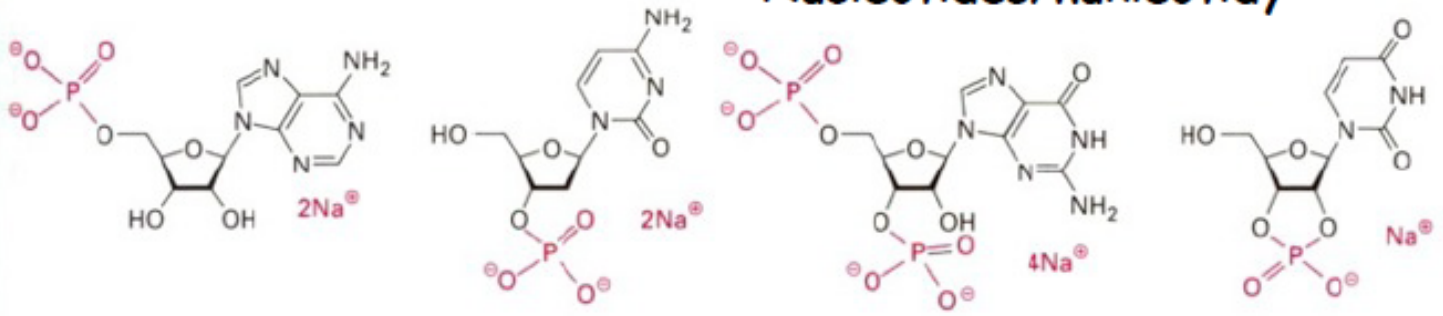
Cytidine
(1-β-D-ribofuranosyl cytosine)



Thymidine
(1-β-D-2-deoxyribo-furanosylthymine)

Thymine deoxyriboside

Nucleotides/nukleotidy



adenosine 5'-phosphate — AMP

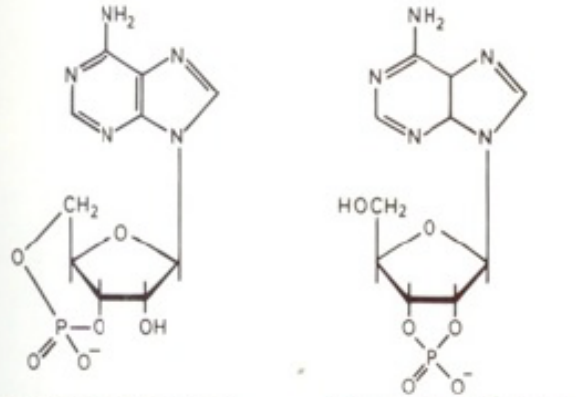
deoxycytidine 3'-phosphate — CMP

guanosine 3', 5'-bisphosphate

uridine 2', 3'-cyclic phosphate — cyclic p cUMP

Fig. 2.4 Structures of some common nucleotides. All are presented as their sodium salts in the state of ionization observed at neutral pH.

Shorthand notation



Adenosine 3′ 5′-cyclic phosphate (cAMP)

Adenosine 2′ 3′-cyclic phosphate

Fig. 2.11

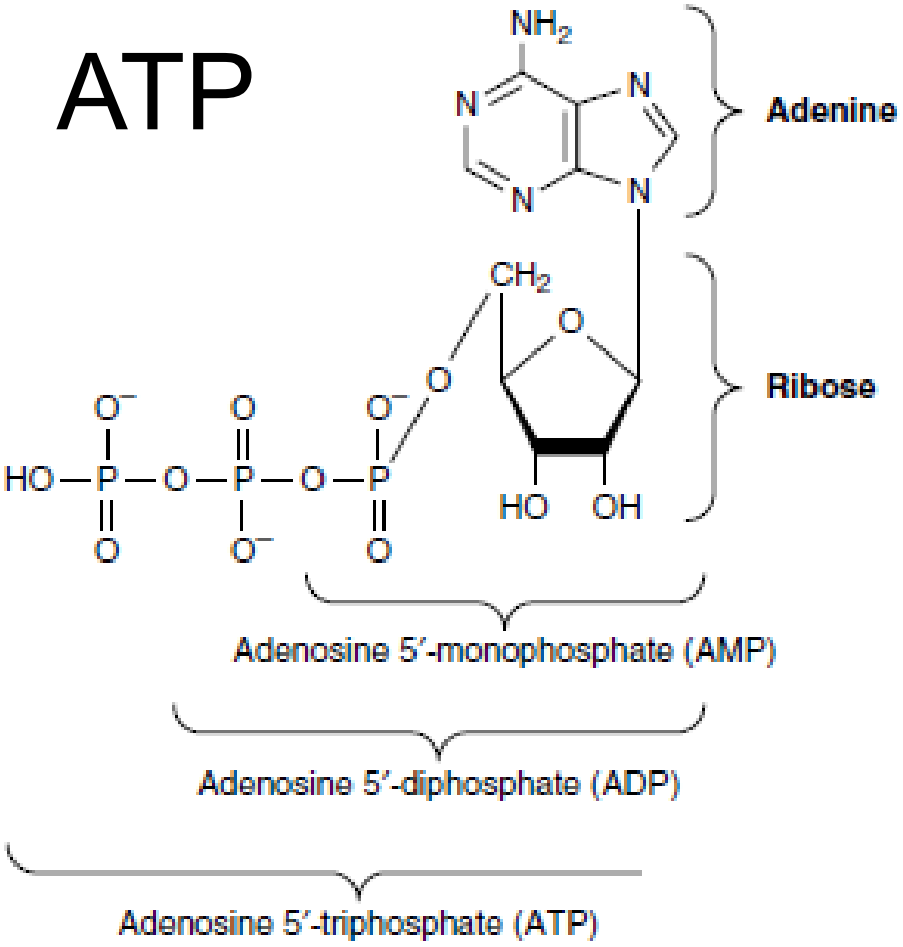
5'-TAGGTCGA-3'
3'-ATCCAGCT-5'

Cp (C-3') x pC (C-5')
UpUp U-Up UpU

bis- x di-phosphates (e.g. ADP)

2 fosfáty na jedné pentose

ATP



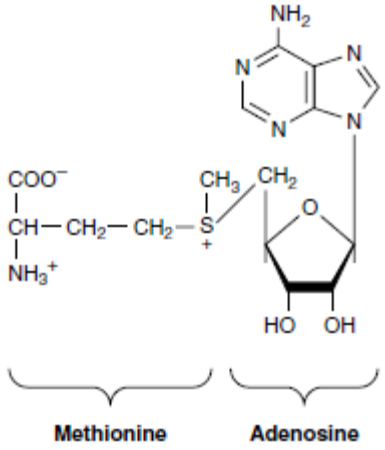
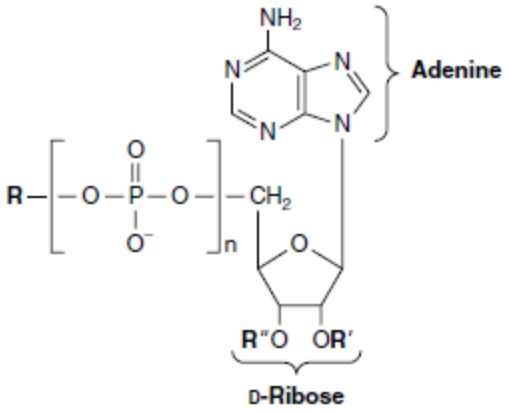


Figure 33–11. *S*-Adenosylmethionine.

Table 33–2. Many coenzymes and related compounds are derivatives of adenosine monophosphate.



Coenzyme	R	R′	R″	n
Active methionine	Methionine*	H	H	0
Amino acid adenylates	Amino acid	H	H	1
Active sulfate	SO_3^{2-}	H	PO_3^{2-}	1
3′,5′-Cyclic AMP		H	PO_3^{2-}	1
NAD*	†	H	H	2
NADP*	†	PO_3^{2-}	H	2
FAD	†	H	H	2
CoASH	†	H	PO_3^{2-}	2

laces phosphoryl group.
a B vitamin derivative.

Neobvyklé báze a nukleosidy

vyskytují se např v tRNA;
jaké další v chromosomálních DNA
(i) prokaryotních a
(ii) eukaryotních buněk?
(iii) v DNA virů?
Může se v DNA vyskytovat uracil?

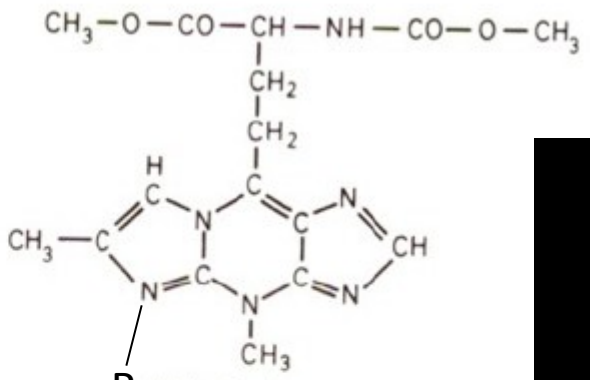
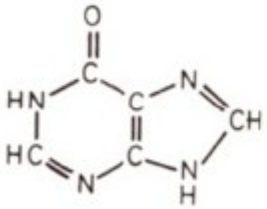
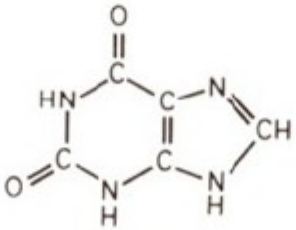


Fig. 2.5

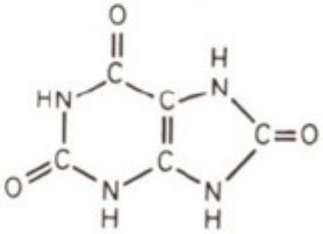
[redacted]e



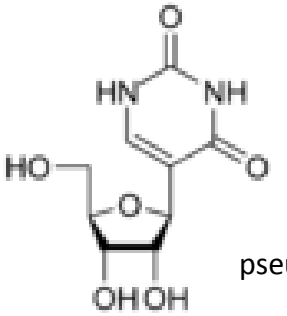
Hypoxanthine
(6-hydroxypurine)



Xanthine
(2,6-dihydroxypurine)



Uric acid
(2,6,8-trihydroxypurine)



pseudouridin

Ionization of AMP

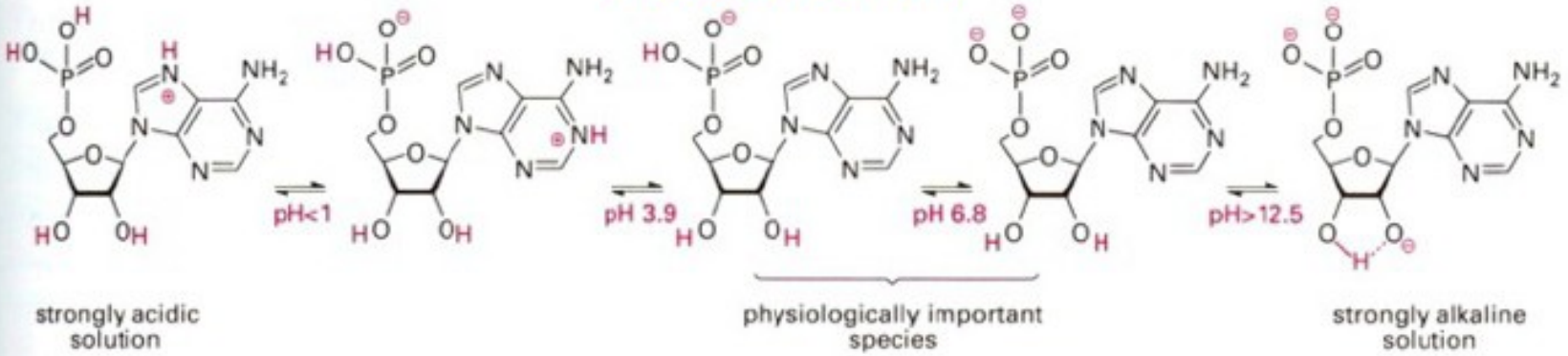


Fig. 2.5 States of protonation of adenosine 5′-phosphate (AMP) from strongly acidic solution (left) to strongly alkaline solution (right).

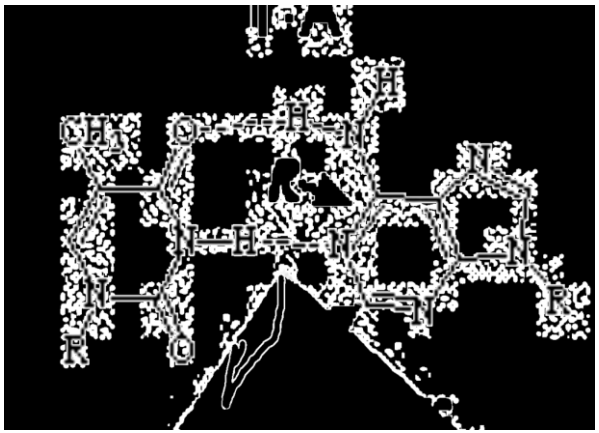
BUT, pK of base residues in DNA may significantly differ!

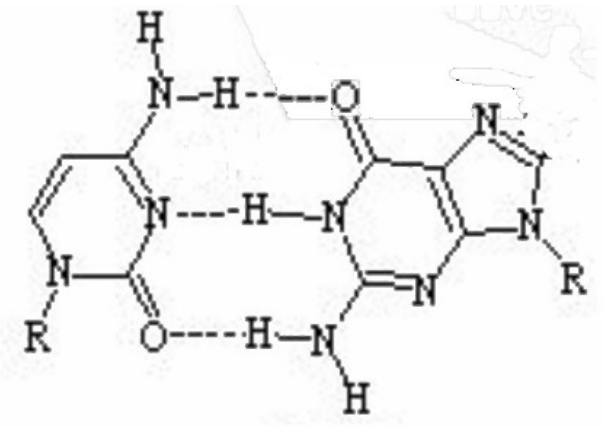
Table 2.1. pK_a values for bases in nucleosides and nucleotides

Base (site of protonation)		*Nucleoside*	*3′-Nucleotide*	*5′-Nucleotide*
Adenine	(N–1)	3.52	3.70	3.88
Cytosine	(N–3)	4.17	4.43	4.56
Guanine	(N–7)	3.3	(3.5)	(3.6)
Guanine	(N–1)	9.42	9.84	10.00
Thymine	(N–3)	9.93	—	10.47
Uracil	(N–3)	9.38	9.96	10.06

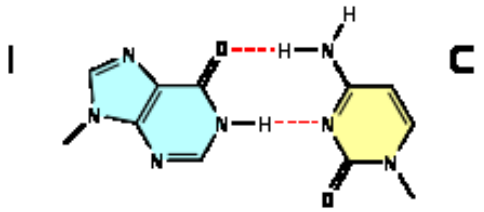
These data relate to 20°C and zero salt concentration. They correspond to *loss* of a proton for $pK_a > 9$ and *capture* of a proton for $pK_a > 5$.

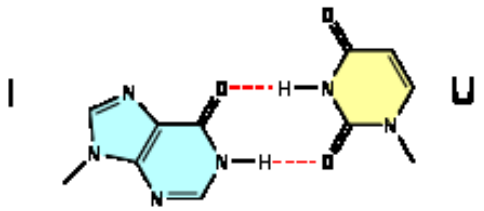
Watsonovy-Crickovy páry bazí (kanonické)

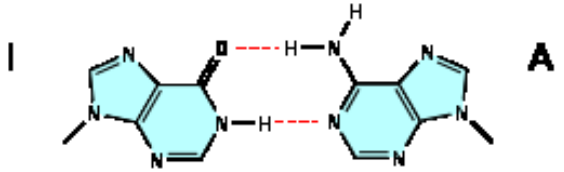


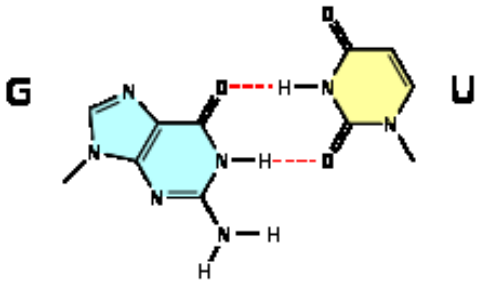


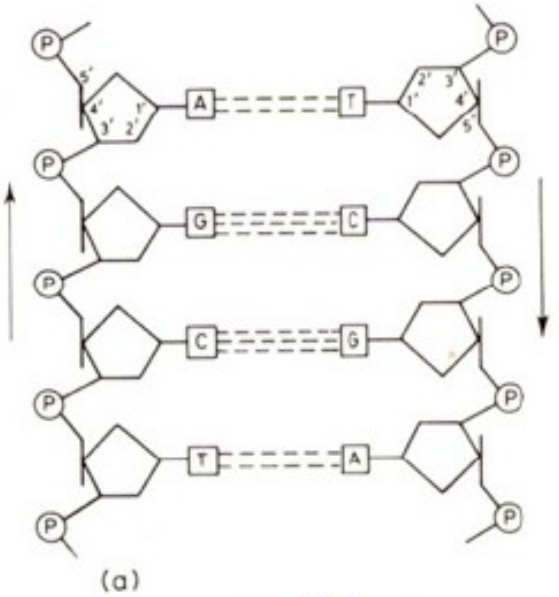
kolísavé (wobble) párování - příklady

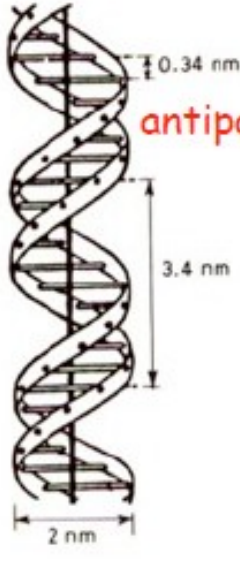












base stacking

antiparallel x parallel strands

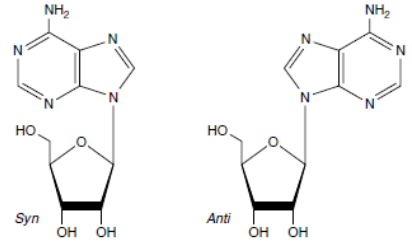
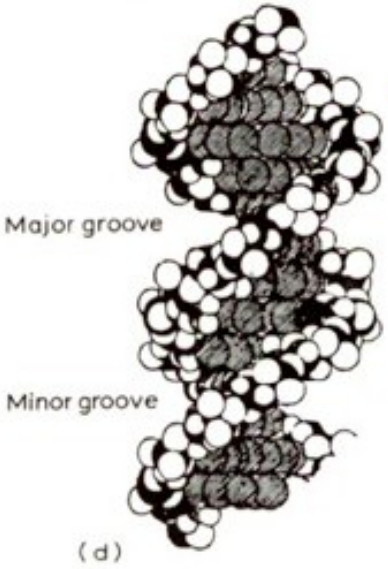


Figure 33–5. The syn and anti conformers of adenosine differ with respect to orientation about the N-glycosidic bond.

Between pH 5 and 9
DNA is a polyanion
with a single negative
charge per nucleotide



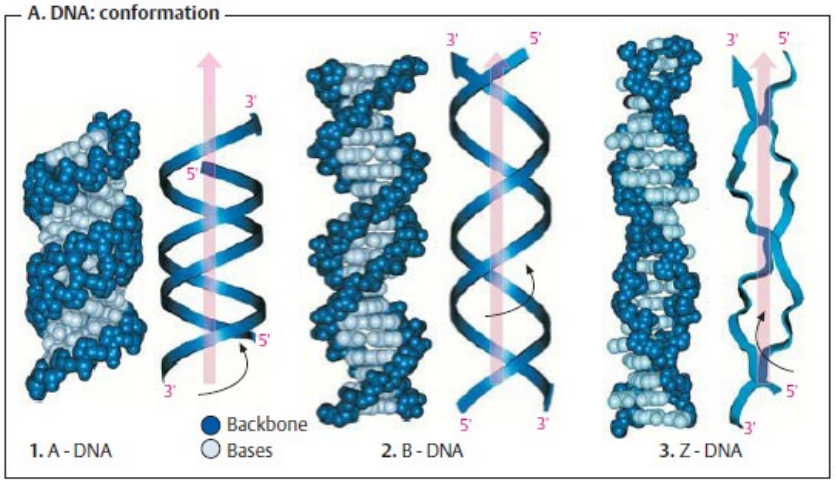
glycosidic bond: ANTI

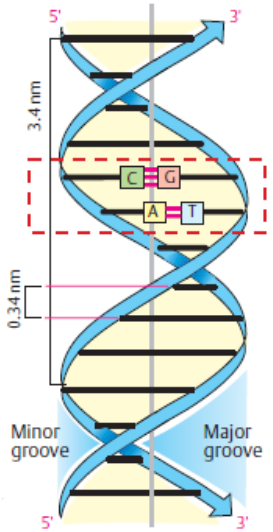
HYDRATION
~70 H_2O/bp

Fig. 2.15 Various diagrammatic ways of representing DNA: (a) showing polarity and base pairing but no helical twist; (b) showing helical twist and helix parameters but not base pairs; (c) showing helix and base pairs; (d) space-filling representation showing major and minor grooves.

TABLE 1
Comparison of A-, B-, and Z-DNA

Helix sense	A-DNA[a] right-handed	B-DNA[a] right-handed	B′-DNA[b] right-handed	Z-DNA[c] left-handed
Base pairs per turn	11	10	10	12 (6 dimers)
Helix twist (°)	32.7	36.0	34.1, 36.8	−10, −50
Rise per base pair (Å)	2.9	3.4	3.5, 3.3	3.7
Helix pitch (Å)	32	34	34	45
Base pair tilt (°)	13	0	0	−7
P distance from helix axis (Å)	9.5	9.3	9.1	6.9, 8.0
Glycosidic orientation	*anti*	*anti*	*anti*	*anti, syn*
Sugar conformation	*C3′-endo*	Wide range	*C2′-endo*	*C2′-endo, C3′-endo*[d]





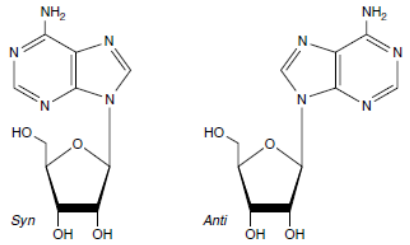
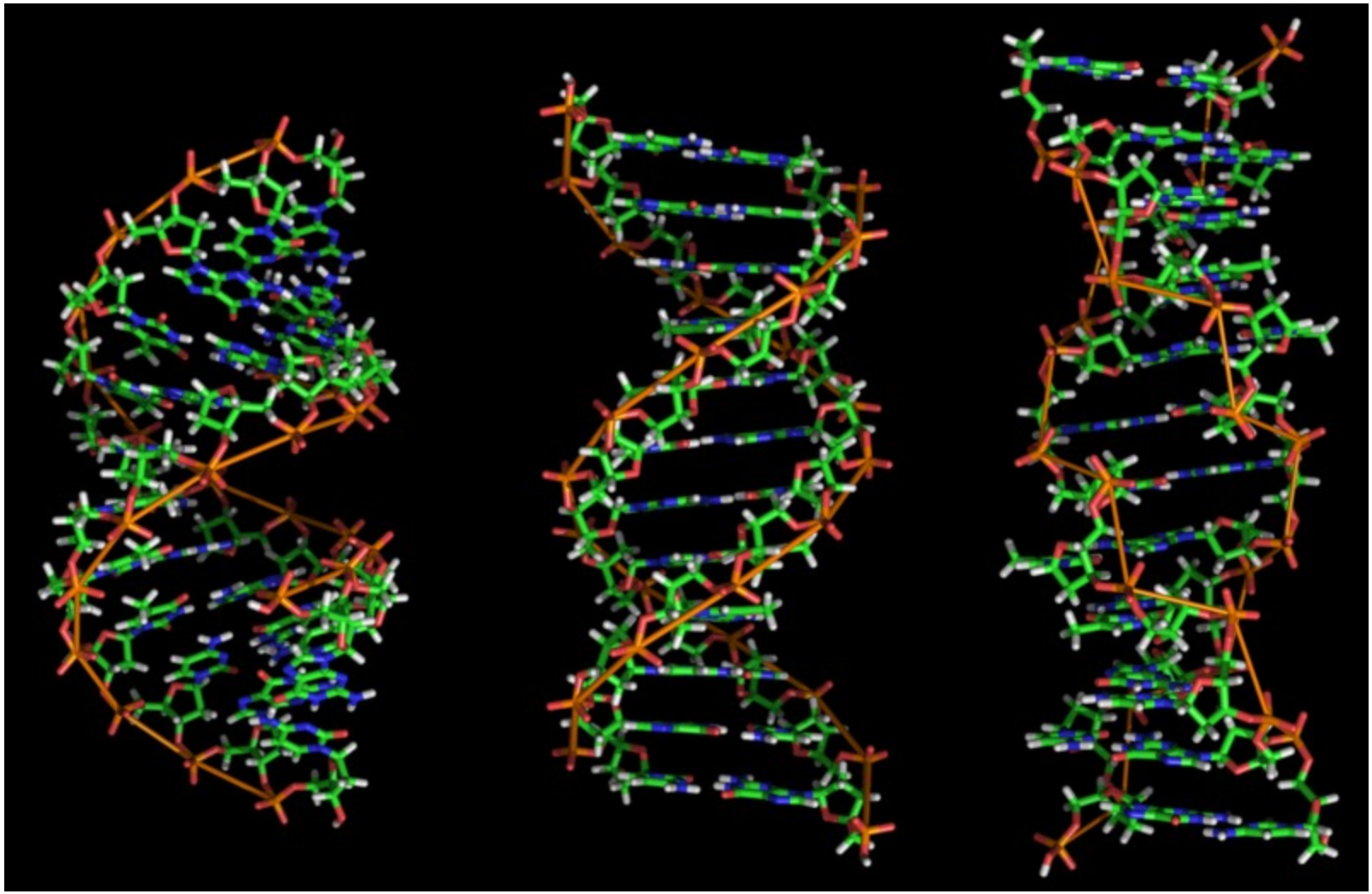


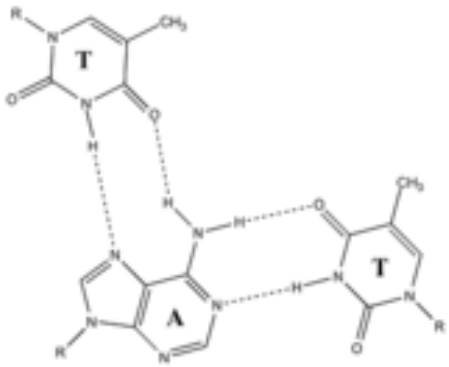
Figure 33–5. The syn and anti conformers of adenosine differ with respect to orientation about the N-glycosidic bond.



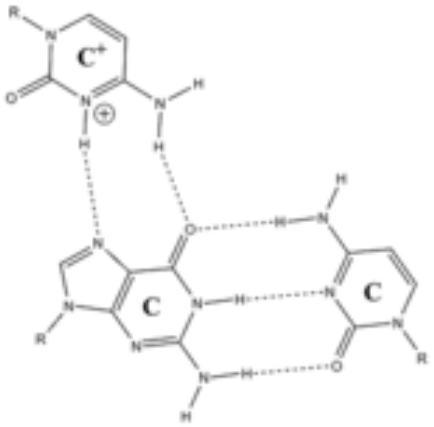
Víceřetězcové struktury DNA

- triplexy
- kvadruplexy

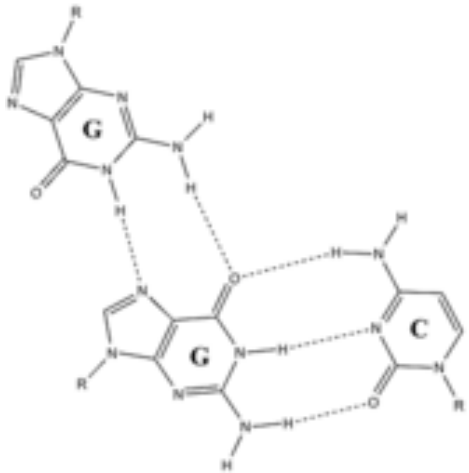
Hoogsteenovo párování (triády bazí)



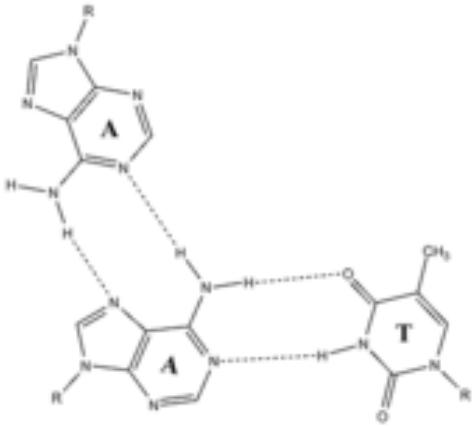
TA*T



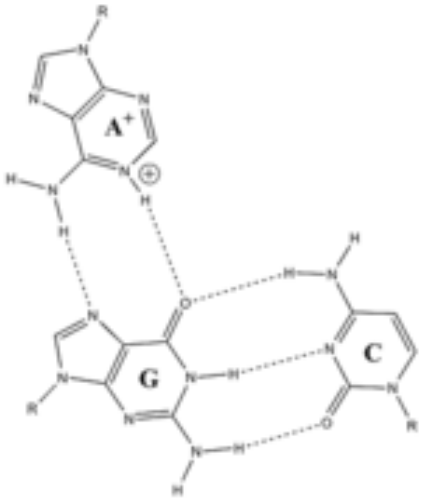
CG*C+



CG*G



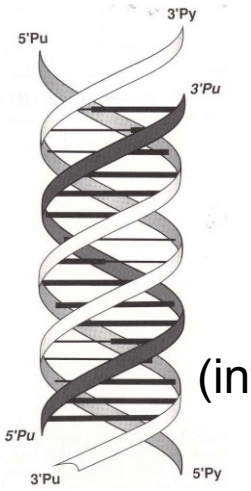
TA*A



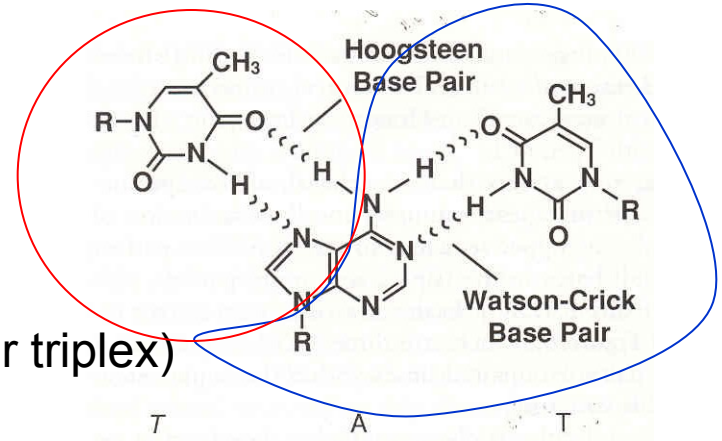
CG*A+

Triplex DNA
(homopurine·homopyrimidine stretch with mirror symmetry)

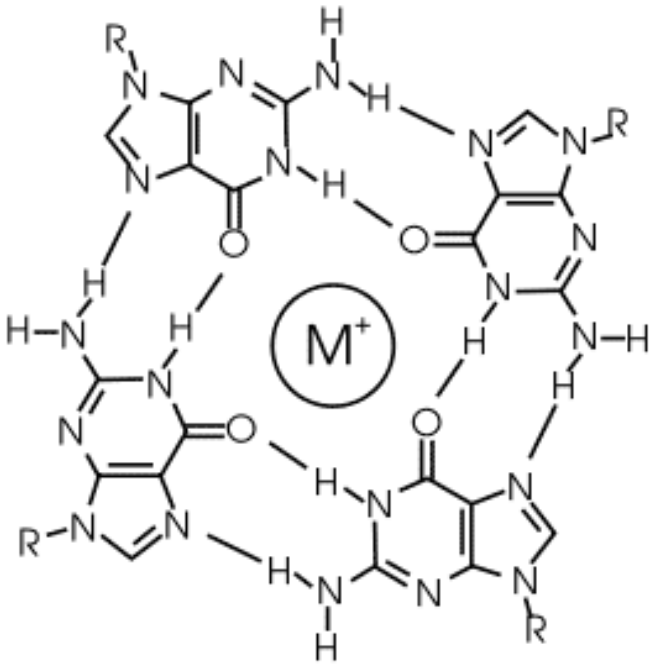
e.g. TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT



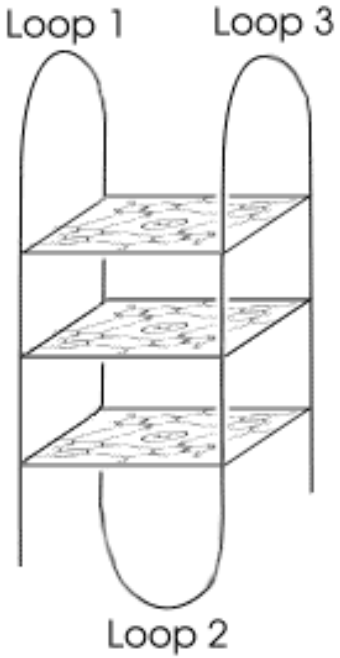
(intermolecular triplex)



Guaninová tetráda



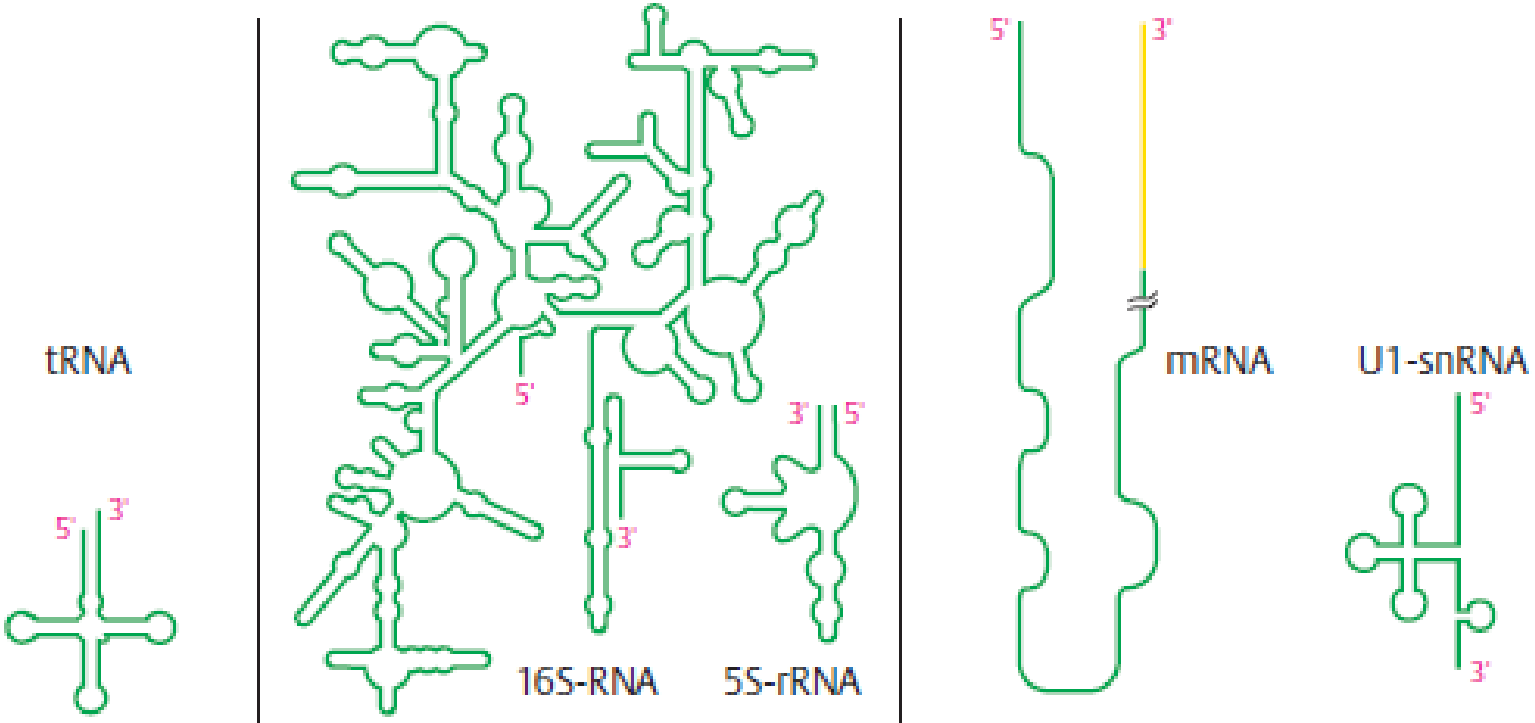
Tetraplex (kvadruplex)



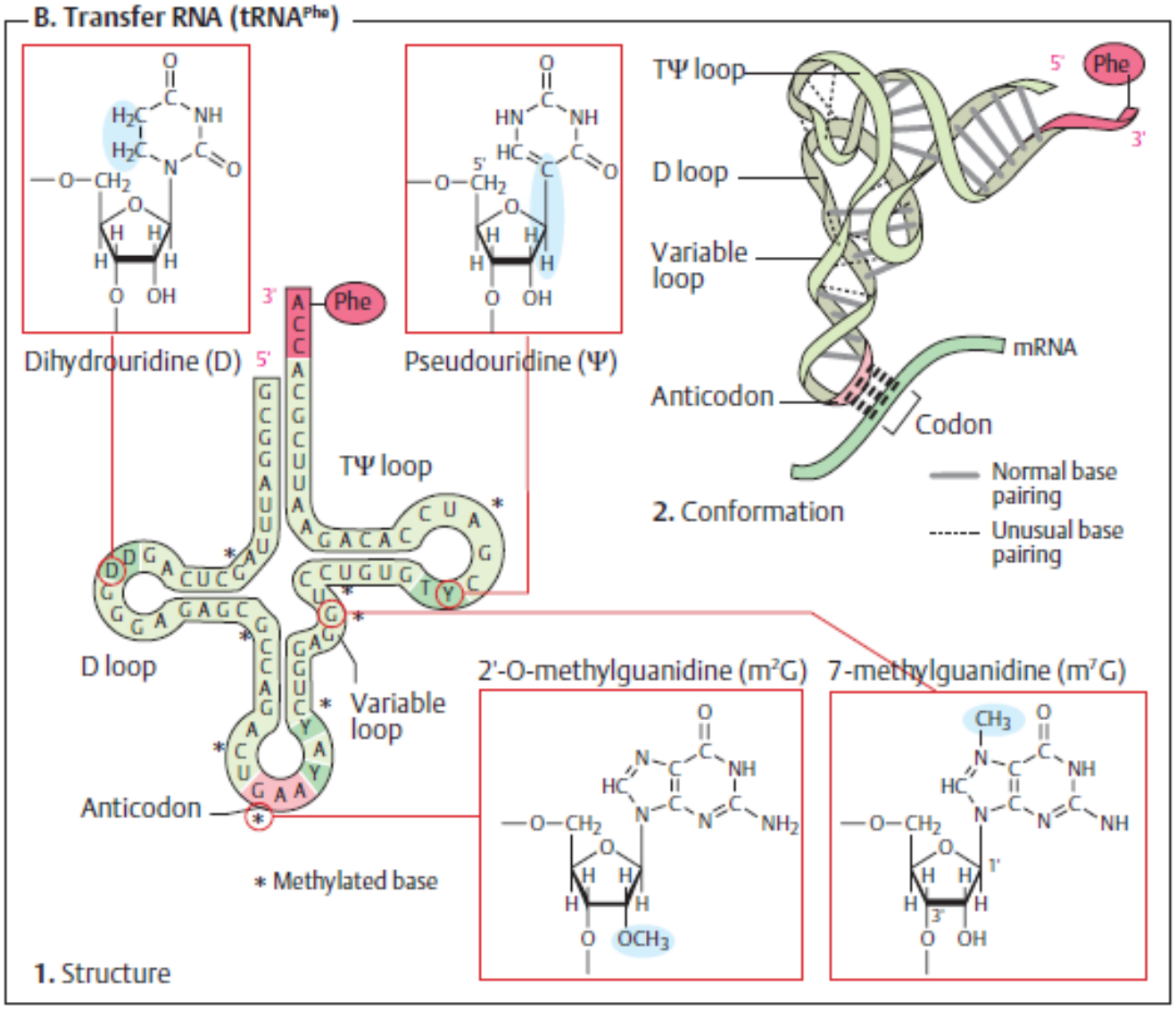
Struktura RNA

- tRNA, rRNA, mRNA… snRNA, siRNA, μRNA…
- transferové a ribozomální RNA: složité terciární (prostorové) struktury
- klíčové funkce (později)

A. Ribonucleic acids (RNAs)

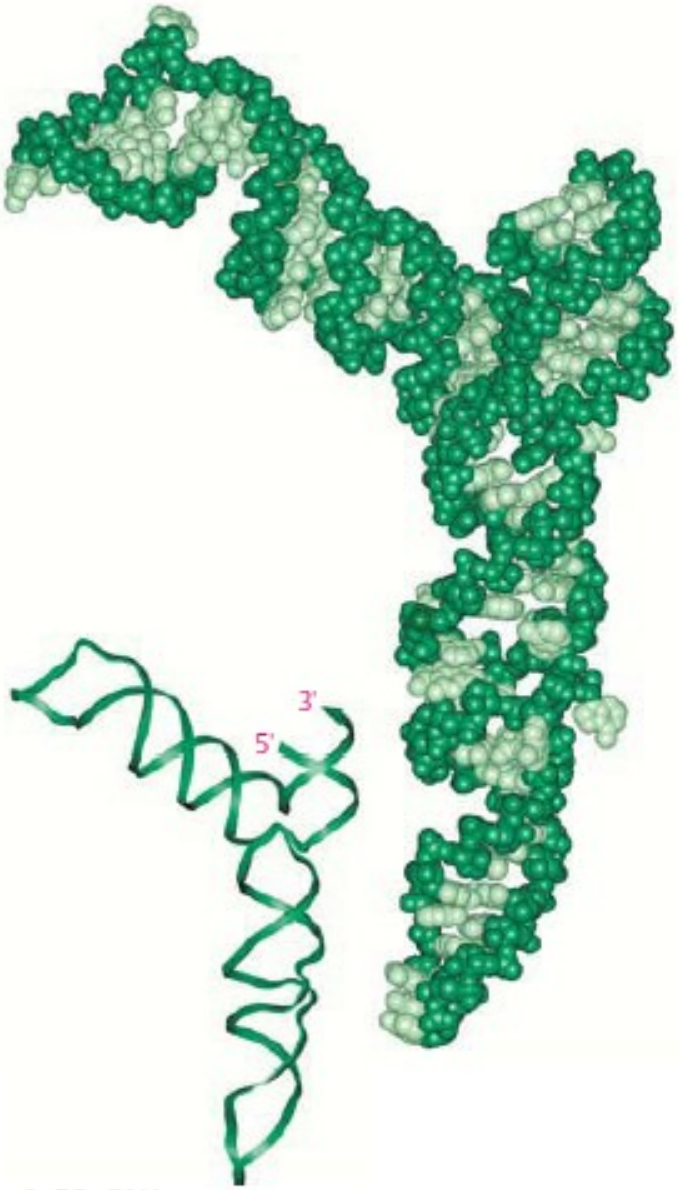


tRNA	rRNA	Type	mRNA	snRNA
>50	4	Species per cell	> 1000	~ 10
74 - 95	120 - 5000	Length (b)	400 - 6000	100 - 300
10-20%	80%	Proportion	5%	< 1%
Long	Long	Lifespan	Short	Long
Translation	Translation	Function	Translation	Splicing

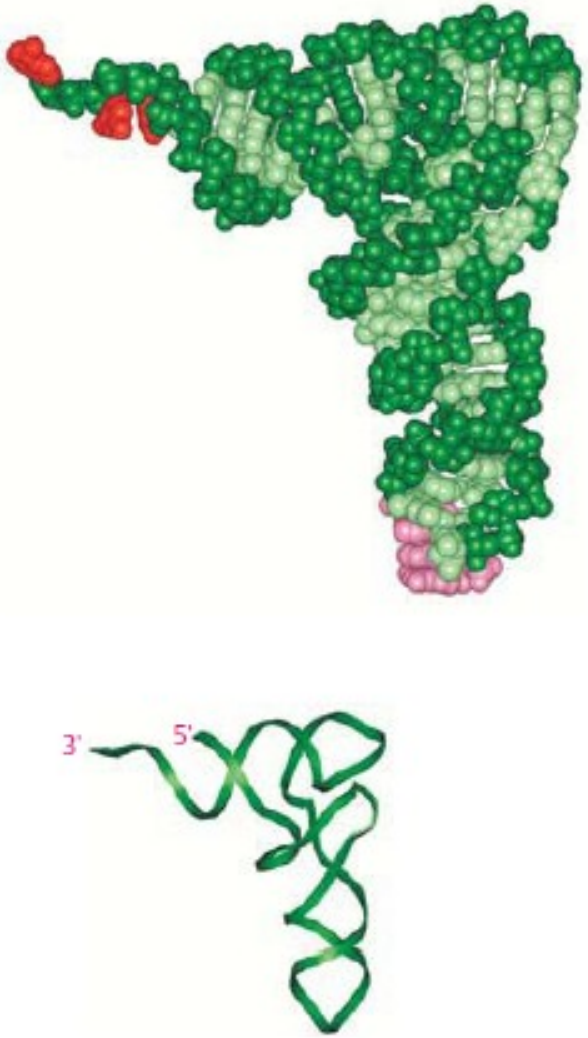


-nekanonické párování
-nejen mezi bázemi
-stabilizuje terciární strukturu

B. RNA

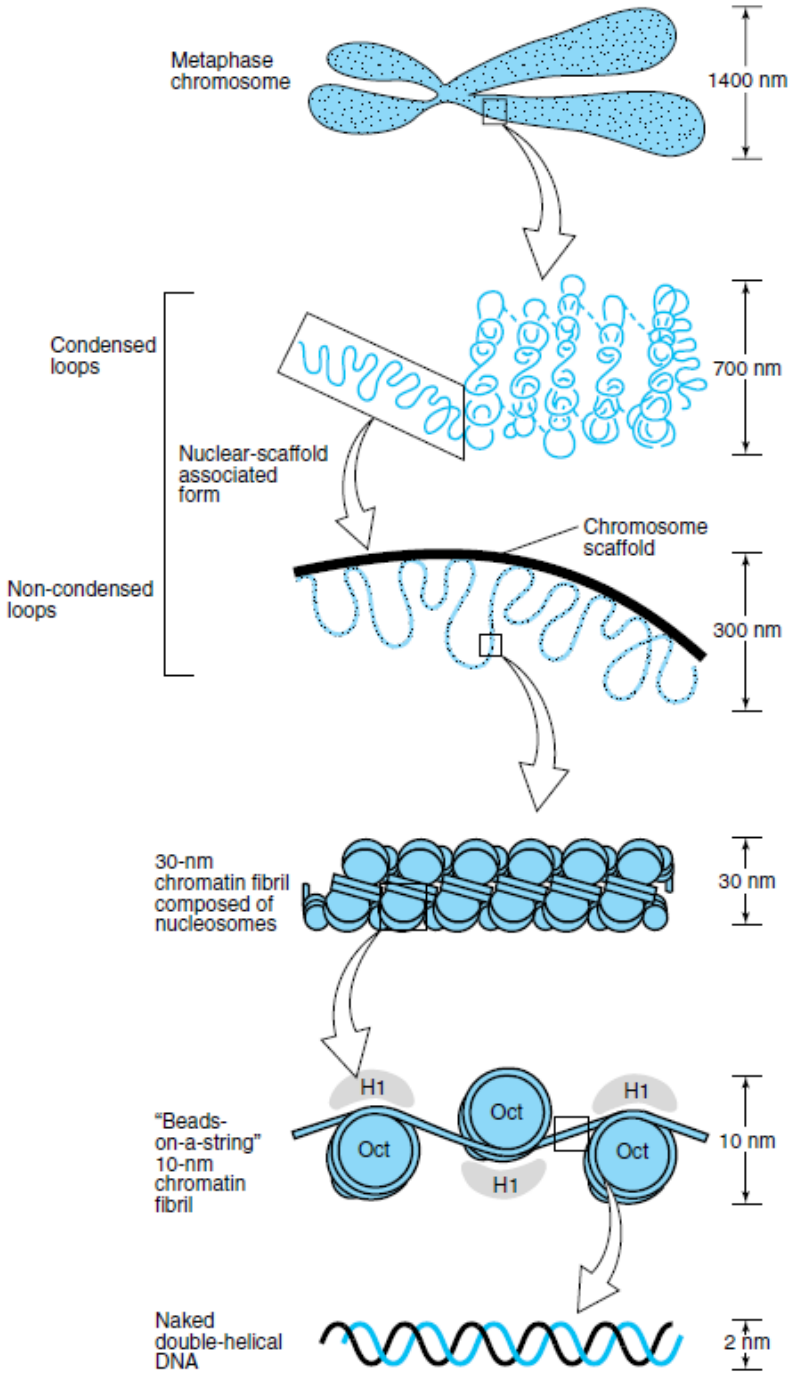


1. 5S-rRNA
(118 nucleotides)

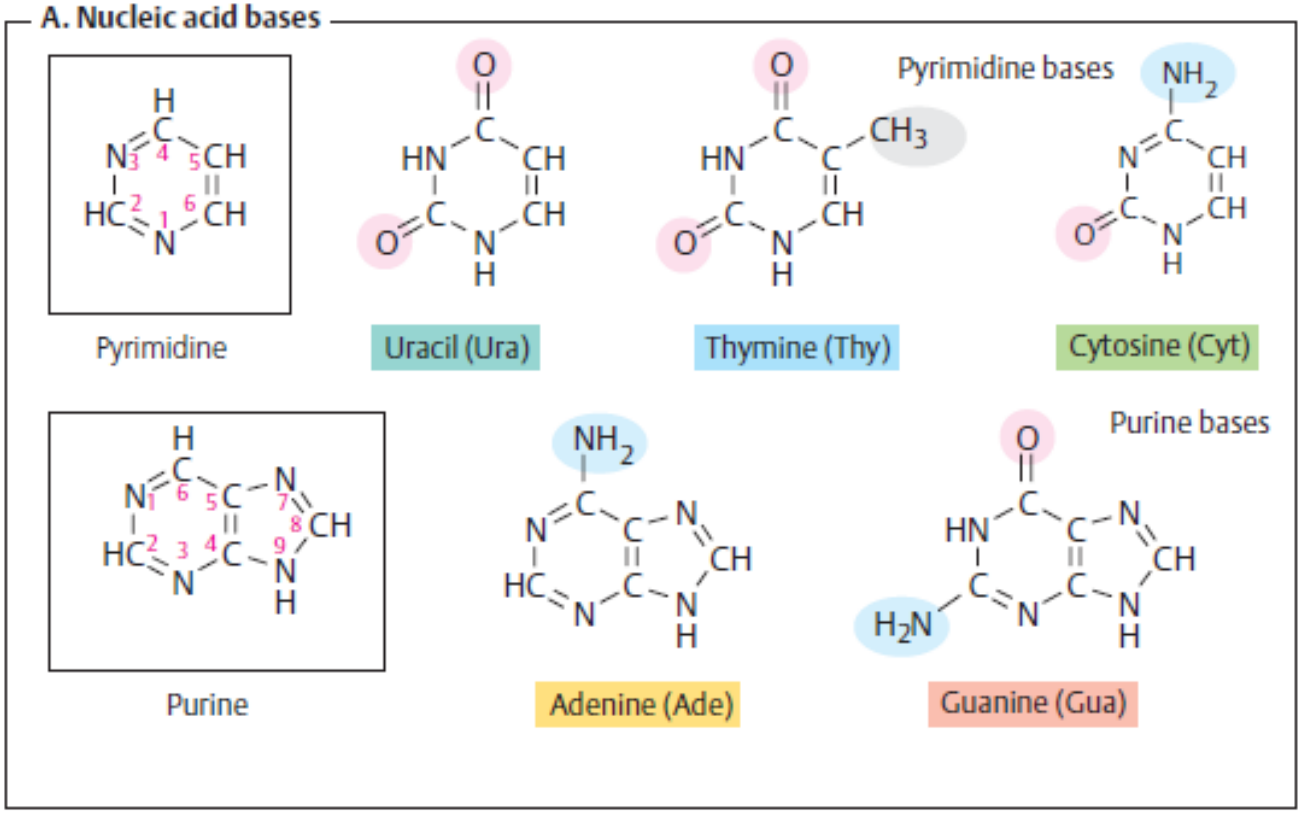


2. Phe-tRNA^{Phe}
(77 nucleotides)

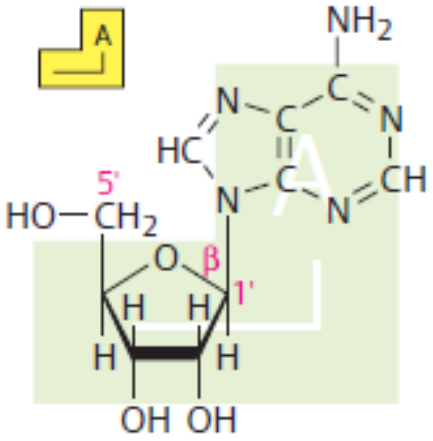
Organizace DNA a chromatinu v buněčném jádře



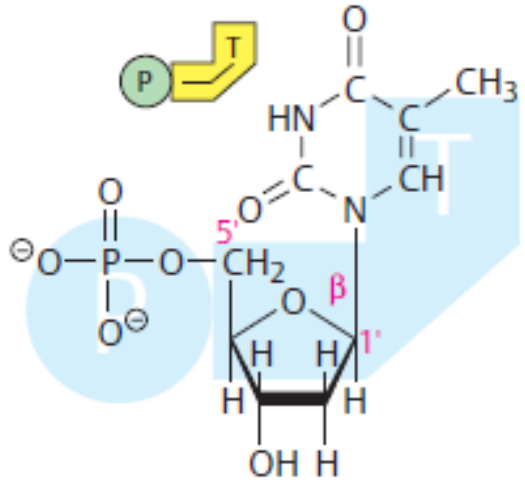
Opakování základních pojmů


A. Nucleic acid bases
Pyrimidine
Pyrimidine bases
Uracil (Ura)
Thymine (Thy)
Cytosine (Cyt)
Purine
Purine bases
Adenine (Ade)
Guanine (Gua)

B. Nucleosides, nucleotides

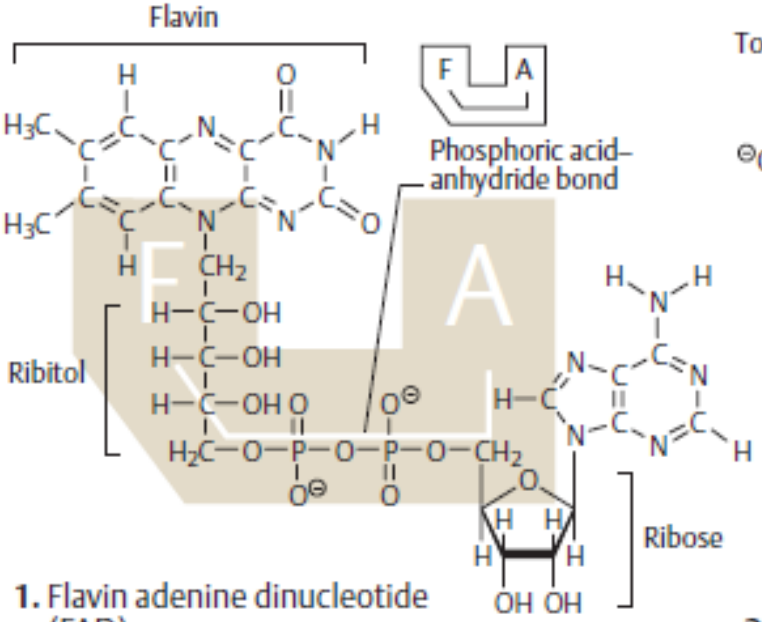


1. Adenosine (Ado)

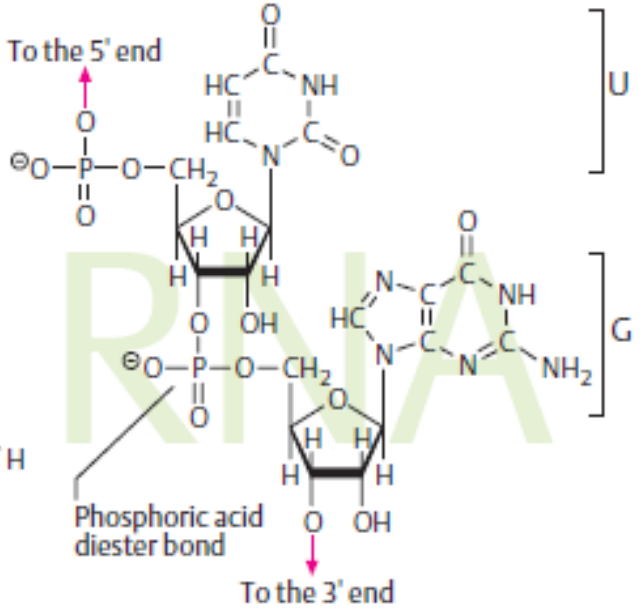


2. 2'-Deoxythymidine 5'-monophosphate (dtMP)

C. Oligonucleotides, polynucleotides



1. Flavin adenine dinucleotide (FAD)



2. RNA (section)

A. DNA: structure